DOI: 10.14744/ejmi.2019.67850
EJMI 2020;4(1):31–35

Research Article

Jak2v617f Mutation in Patients with Myeloproliferative Diseases and Those with Ischemic Heart Disease with Normal Coronary Angiography

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Abstract

Objectives: Arterial and venous thrombotic events are more commonly observed in patients with myeloproliferative diseases (MPDs). Arterial and venous thromboses are significant causes of mortality and morbidity in Philadelphia (Ph) chromosome-negative MPDs. The present study investigates the presence of the JAK2V617F mutation, which contributes to the early diagnosis of MPD, in patients with ischemic heart disease with a normal coronary angiography and in those with a venous thrombosis in an atypical location. The goal in this regard is to determine the contribution of the JAK2V617F mutation to the development of thrombosis in Philadelphia chromosome-negative MPDs, and to identify possible relationships between the JAK2 mutation and other clinical and laboratory characteristics.

Methods: The study was conducted in the Division of Hematology of the Department of Internal Medicine in the Trakya University Faculty of Medicine between March 2008 and August 2009. Approval for the study was granted by the Ethics Committee of the Trakya University Faculty of Medicine on February 21, 2008, and the study was supported by the Trakya University Scientific Research Projects Fund. A total of 87 subjects were included in the study. The JAK2V617F mutation was analyzed using a real-time PCR device and with a melting curve analysis. The demographic and medical data of the patients was retrieved from the medical charts. The statistical analysis was performed using SPSS 13.0 data analysis software.

Results: The study included 31 patients diagnosed with myeloproliferative disease, 32 patients with ischemic heart disease with a normal coronary angiography and four patients with a venous thrombosis in an atypical location, as well as 20 healthy volunteers included in the control group. The JAK2V617F mutation was identified in 24 patients (77.4%) with myeloproliferative disease, in one patient (3.1%) with cardiovascular disease and in two patients (50%) with a venous thromboembolism in an atypical location. No JAK2V617F mutation was found in the healthy control group. There was no difference between myeloproliferative disease subgroups in terms of history of thrombosis. No significant relationship was found between the presence of the JAK2V617F mutation, history of thrombosis and leukocyte count, or between leukocyte count and history of thrombosis (p=0.183, p=0.345, p=0.368, respectively).

Conclusion: The identification of the JAK2V617F mutation in three patients with thrombosis with no diagnosis of myeloproliferative disease suggests that the detection of this mutation in patients with a venous thromboembolism in an atypical location and in some patients with an arterial thrombosis may contribute to the early recognition of patients with myeloproliferative disease, and may give these patients the chance to begin the appropriate therapy. The rate of a history of thrombosis was higher in the JAK2V617F-positive patients, although the difference did not reach statistical significance.

Keywords: Coronary thrombosis, mutation, myeloproliferative diseases, thrombosis

Cite This Article: *Yalcin Musri F, Turgut B, Musri OC, Isik S, Karakurt Eryilmaz M. Jak2v617f Mutation in Patients with Myeloproliferative Diseases and Those with Ischemic Heart Disease with Normal Coronary Angiography. EJMI 2020;4(1):31–35.*

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Submitted Date: November 15, 2019 **Accepted Date:** December 18, 2019 **Available Online Date:** January 17, 2020

Myeloproliferative diseases (MPDs) are hematopoietic stem cell diseases that are characterized by a proliferation of the myeloid cell line with no interruption in differentiation or maturation. According to the classification of the World Health Organization (WHO), chronic myeloid leukemia (CML), polycythemia vera (PV), primary myelofibrosis (PMF) and essential thrombocytopenia (ET) are all chronic MPDs.[1] It is known that increased tyrosine kinase activity associated with the bcr-abl fusion gene that results from the t(9;22) translocation known as the Philadelphia (Ph) chromosome plays a role in the pathogenesis of CML. It was shown in 2005 that increased kinase activity plays a role in such Ph-negative MPDs as PV, MF and ET. A point mutation has been identified in the Janus kinase 2 (JAK2) gene that results in an increased kinase activation.[2–5] Previous studies have demonstrated the presence of the JAK2V617F mutation in more than 95% of cases with PV, and in 50–60% of cases with ET and PMF.[5-9] Janus kinase 2 is a cytoplasmic tyrosine kinase that is involved in the signal transduction of such important growth factors as erythropoietin and thrombopoietin, both in normal and neoplastic cells.[10] A G-T transversion at nucleotide 1.849 in exon 14 of the JAK2 gene leads to the substitution of phenylalanine for valine (V617F) at codon 617 of the JAK2 protein. This mutation results in the uncontrolled activation of the Janus kinase/signal transducers and the activators of the transcription (JAK/STAT) signaling pathway, which is one of the key components in cellular growth and differentiation.[11] Arterial and venous thrombotic events are more commonly observed in patients with myeloproliferative diseases. Arterial and venous thromboses are important causes of mortality and morbidity in Ph-negative MPDs.[12] Of the thrombotic events occurring in MPDs, 75% are observed in the arteries, and thrombotic occlusions of the arteries often affect the cerebral and coronary arteries.[13] Venous thromboembolism in MPDs most often manifests as a deep venous thrombosis and pulmonary embolism, although venous thromboses in an atypical location specifically affect this group of patients.

Coronary artery disease is one of the most frequent causes of death. Thrombosis associated with atherosclerosis constitutes the basis of the etiopathogenesis in these patients. Thrombosis observed in the coronary arteries without underlying atherosclerosis suggests that some may be associated with MPDs, the classical findings of which have yet to become apparent. The present study investigates the presence of the JAK2V617F mutation, which contributes to the early diagnosis of myeloproliferative diseases, in patients with ischemic heart disease with a normal coronary angiography, and in patients with a venous thrombosis in an atypical location.

Methods

The study was conducted in the Division of Hematology of the Department of Internal Medicine in the Trakya University Faculty of Medicine between March 2008 and August 2009. All patients included in the study signed a written informed consent form, providing information to the patient about the study and documenting the patient's granting of informed consent. Approval for the study was granted by the Ethics Committee of Trakya University Faculty of Medicine on February 21, 2008 (Protocol code: TÜFTEK 2008/13). The study was supported by the Trakya University Scientific Research Projects Fund (Project No: TÜBAP 2008-51). A total of 87 subjects were included in the study, divided into four groups. The first group was composed of 31 patients with MPDs, including 12 patients with PV, 13 patients with ET and six patients with PMF who were followed up in the Division of Hematology. The second group was composed of 32 patients who were diagnosed with IHD, despite normal findings on a coronary angiography performed in the Department of Cardiology of the Trakya University Faculty of Medicine. The third group was composed of four patients who were diagnosed with a venous thrombosis in an atypical location and followed up in the Division of Hematology of the Department of Internal Medicine of the Trakya University Faculty of Medicine. Finally, 20 healthy subjects with no known medical condition were included as a control group (fourth group). The JAK2V617F mutation was analyzed using a real-time PCR device and melting curve analysis, and the presence of a JAK2 mutation was defined as either homozygous or heterozygous. The demographic and medical data of the patients was obtained from a review of medical files. The SPSS version 13.0 data analysis software was used for the statistical analysis. A p value of less than 0.05 was considered statistically significant in all analyses.

Results

A total of four groups were created, including a control group. A comparison of the groups in terms of age and gender showed a significant difference in age between the groups (p=0.014), whereas no significant difference was noted in gender (p=0.428). The patients with a venous thrombosis in an atypical location were not included in the statistical comparison due to their small number (Table 1).

Past medical history was remarkable for thrombosis in six out of 31 patients (19.4%) in the MPD group. Among the subgroups, the rate of thrombosis was highest in patients with PMF (50%). No significant difference was found between the subgroups in a statistical comparison due to the small number of patients (p=0.097). No statistically significant difference was noted among the subgroups of

Table 1. Comparison of groups in terms of age and gender

	Group 0 n=20	Group 1 n=31	Group 2 n=32	Group 3 n=4	*p
Age (years)	52.0±6.90	59.38±13.16	53.15±7.19	51.50±18.80	0.014
Gender, Female (n, %)	12 (60)	13 (41.9)	17 (53.1)	3 (75)	0.428

The results are presented as mean±SD. *All groups other than Group 3 were compared.

Table 2. Demographic and clinical characteristics of myeloproliferative disease subgroups

	PV, n=12 (%)	PMF, n=6 (%)	ET, n=13 (%)	Total, n=31 (%)	p
Cytoreductive therapy	9 (75)	5 (83.3)	13 (100)	27 (87.1)	*
History of thrombosis	1 (8.3)	3 (50)	2 (15.4)	6 (19.4)	0.097
Smoking	4 (33.3)	0	3 (23.1)	7 (22.6)	0.28
Acetylsalicylic acid	12 (100)	4 (66.7)	13 (100)	29 (93.5)	*
Splenomegaly	7 (58.3)	5 (83.3)	8 (61.5)	20 (64.5)	*
Hepatomegaly	2 (16.7)	5 (83.3)	2 (15.4)	9 (29)	*
Hemoglobin (g/dl)	16.8±2.05	11.1±3.05	12.7±2.06	14.0±3.2	*
Leukocyte (/πl)	12.248.3±8.426.2	32.775.00±48.201.8	14.123.07±5.736.6	17.007.4±22.109.3	*
Platelet (/πl)	446.083±251.928	556.000±644.804	961.692±297.877	683.580±432.889	*

The results of the complete blood count are presented as mean±SD. *No statistical comparison has been made.

patients with MPD in terms of smoking (p=0.28). The clinical and demographic data pertaining to the subgroups of patients with MPD is presented in Table 2.

Of the 32 patients (34.4%) in the cardiovascular disease group, 11 were smokers and 19 (59.4%) were taking acetylsalicylic acid. The clinical and demographic characteristics of the patients in the cardiovascular disease group are presented in Table 3.

No JAK2 mutation was identified in any of the subjects in the control group, as expected. A heterozygous mutation was found in one patient (3.1%) in the CVD group. Of the four patients with a venous thrombosis in an atypical location, two (50%) were heterozygous for the JAK2V617F mutation. The JAK2V617F mutation was also identified in 24 of the 31 patients (77.4%) in the MPD group. Of these patients, 23 patients were heterozygous and one patient with PFM was homozygous for the mutation. The JAK2V617F mutation was identified in nine out of 12 patients with polycythemia vera (75%), 10 out 13 patients with ET (76.9%), and 5 out of 6 patients with PMF (83.4%). No statistically significant difference was noted between myeloproliferative disease subgroups in terms of the frequency of JAK2V617F mutation (p=0.922), possibly due to small number of the parameters (Table 4).

A history of thrombosis was remarkable in six (19.4%) out of the 24 (77.4%) patients in the MPD patient group who were carriers of the JAK2V617F mutation. A history of thrombosis was unremarkable in patients with MPDs without JAK2V617 mutations. One patient in the PMF group who was homozygous for the mutation had deep vein thrombosis in the lower extremities, and the other two patients had coronary artery thrombosis – one with a thrombosis in the PV subgroup who had coronary artery thrombosis, and one in the ET group who had coronary artery thrombosis, while the other had cerebral artery thrombosis. One patient had venous and five patients had arterial thrombosis in the MPD group, and of the patients with arterial thrombosis, four had coronary and one had cerebral artery thrombosis. A statistical analysis of the relationship between the presence of the JAK2V617 mutation in MPD disease and a history of thrombosis showed no statistically significant relationship between the two parameters (p=0.183) (Table 5).

Table 3. Demographic and clinical characteristics of patients in the cardiovascular disease group

	Cardiology patients, n=32 (%)
Smoking	11 (34.4)
Acetylsalicylic acid	19 (59.4)
Hypertension	11 (34.4)
Diabetus mellitus	8 (25)
Hyperlipidemia	1 (3.1)
Hemoglobin (g/dl)	13.6±1.5
Leukocyte (/πl)	8.053.1±1.715.5
Platelet (/πl)	264.531.2±59.058.7

The results of the complete blood count are presented as mean±SD.

Table 4. Presence of JAK2V617F mutation in the myeloproliferative disease subgroups

	PV n=12	PMF n=6	ET n=13	Total n=31	p
JAK2, n (pozitive %)	9 (75)	5 (83.4)	10 (76.9)	24 (77.4)	0.922

Table 5. Comparison of JAK2V617F and history of thrombosis in the myeloproliferative disease subgroup

	Thrombosis history present, n (%)	Thrombosis history absent, n (%)	p
JAK2V617F-positive	6 (19.4)	18 (58.1)	0.183
JAK2V617F-negative	0	7 (22.6)	

An analysis of the relationship between the presence of JAK2V617F mutation and leukocyte count in the myeloproliferative disease group showed no statistically significant relationship between the two parameters (p=0.345). There was also no statistically significant relationship identified between leukocyte count and thrombosis in the same group (p=0.368).

Discussion

A heterozygous JAK2V617F mutation was identified in one of the 32 patients with IHD with normal coronary arteries, and two out of the four patients with an atypical VTE. The frequency of the JAK2V617F mutation was found to be high in Ph-negative patients with MPD, as expected. The primary objective of the study was to investigate the relationship between the JAK2V617F mutation and thrombosis. For this reason, identification of this mutation in three patients with no previous diagnosis of MPD is an important finding, suggesting that thromboembolic events may be associated with a yet undiagnosed MPD and that the identification of the JAK2V617F mutation may reveal such cases. Among the patients with MPD, the JAK2V617F mutation was identified in nine patients with PV (75%), 10 patients with ET (76.9%) and five patients with PMF (83.4%). All JAK2V617F mutations were heterozygous, aside from in one patient in the PMF group. The frequency of the JAK2V617F mutation has been reported to be higher than 95% in patients with PV and 50-60% in patients with ET and PMF.[5-9] The frequency of the JAK2V617F mutation among patients with PV in the present study was lower than expected, but higher than expected in the other two MPDs. The reason for this might be the insufficient number of patients included in the study.

There is a well-known predisposition to arterial and venous thrombosis in Philadelphia-negative MPDs.[14] Although a large number of mechanisms have been proposed to explain the role of Ph-negative MPDs in the pathogenesis of thrombosis, recent studies have suggested that leukocyte count and JAK2V617F allele load are related to the risk of thrombosis.[15] The present study evaluated the relationship between leukocyte count and thrombosis, and found considerably higher mean leukocyte counts in six patients with MPD and with a history of thrombosis (32.725/µl) than in 25 patients with MPD without a history of thrombosis (13.235/µl). Despite this difference, no statistically significant relationship was identified between leukocyte count and thrombosis due to the small number of patients with possible thrombosis. Recent studies have focused on the relationship between leukocyte count and JAK2 allele load in MPDs.[16–18] The present study evaluated the relationship between the JAK2V617F mutation and the leukocyte count, but could identify no statistically significant relationship due to small number of cases. The mean leukocyte count was higher in JAK2V617F-positive patients (18.512/µl) than in patients testing negative for the mutation (11.848/µl). In our study, the history of thrombosis was remarkable in six (5 had arterial and 1 had venous) out of the 24 Ph-negative patients with MPD who tested positive for the JAK2V617F mutation. However, seven JAK2V617F-negative patients with MPD had no history of thrombosis. This striking finding, however, could not be supported statistically due to the small number of cases. The JAK2V617F mutation was identified in one out of 32 patients with IHD and in a normal coronary angiography. The clinical and laboratory findings in this patient was not sufficiency to diagnose any of negative MPDs. In conclusion, a heterozygous JAK2V617F mutation was identified in one patient with ischemic heart disease with a normal coronary angiography, and in two patients with an atypical VTE and with no previous diagnosis of MPD. On the other hand, the frequency of JAK2V617F positivity was considerably high in patients with MPDs, as could be expected, and a history of thrombosis was remarkable in JAK2V617F-positive patients, although not to a statistically significant level. Studies involving a larger series of patients are required to confirm the findings of the present study.

Disclosures

Ethics Committee Approval: The Ethics Committee of Trakya University Faculty of Medicine provided the ethics committee approval for this study (21.02.2008-04/10).

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – F.Y.M., B.T. ; Design – F.Y.M., B.T.; Supervision – F.Y.M., B.T.; Materials – F.Y.M., B.T.; Data collection &/or processing – F.Y.M., Ö.C.M.; Analysis and/or interpretation – F.Y.M., B.T.; Literature search – F.Y.M., Ö.C.M.; Writing – F.Y.M., Ö.C.M., S.I. ; Critical review – F.Y.M., M.K.E.

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